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Research paper

Daily and seasonal dynamics of remotely sensed photosynthetic efficiency in tree canopies

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The photosynthesis of various species or even a single plant varies dramatically in time and space, creating great spatial heterogeneity within a plant canopy. Continuous and spatially explicit monitoring is, therefore, required to assess the dynamic response of plant photosynthesis to the changing environment. This is a very challenging task when using the existing portable field instrumentation. This paper reports on the application of a technique, laser-induced fluorescence transient (LIFT), developed for ground remote measurement of photosynthetic efficiency at a distance of up to 50 m. The LIFT technique was used to monitor the seasonal dynamics of selected leaf groups within inaccessible canopies of deciduous and evergreen tree species. Electron transport rates computed from LIFT measurements varied over the growth period between the different species studied. The LIFT canopy data and light-use efficiency measured under field conditions correlated reasonably well with the single-leaf pulse amplitude-modulated measurements of broadleaf species, but differed significantly in the case of conifer tree species. The LIFT method has proven to be applicable for a remote sensing assessment of photosynthetic parameters on a diurnal and seasonal scale; further investigation is, however, needed to evaluate the influence of complex heterogeneous canopy structures on LIFT-measured chlorophyll fluorescence parameters.

Keywords: electron transport rate, fluorescence, LIFT, PAM, photosynthesis, remote sensing.

Introduction

Modulation of the photosynthetic processes by biological and environmental factors spans a large temporal range from seconds in fluctuating light conditions to very slow seasonal changes (Rascher and Nedbal 2006, Schurr et al. 2006). Consideration of photosynthesis at the scale of ecosystems or the entire globe implies integration of these temporal variations over many individual elements distributed over a heterogeneous spatial environment. In the field, the most frequently used approaches to monitor plant photosynthetic performance on the ecosystem scale are based on the eddy-covariance method (Baldocchi 2003) or remote sensing observations (Hilker et al. 2008). These bulk measurements of the exchange processes of the entire ecosystem are often based on many assumptions. Therefore, they provide little understanding of the underlying processes on the scale of single leaves or branches in the plant canopy under field conditions.

Plant canopies are exposed to a substantial gradient in light from the top of the canopy to the bottom, which affects the temperature and humidity and thus vapor pressure deficit within different layers of the canopy. Leaves within these different layers acclimate to these conditions, e.g., by different light-harvesting efficiencies in low or high light (Niinemets 2007) or redistribution of nitrogen (Field et al. 1995). Leaves of different

species and in different canopy positions often respond differently to the prevailing and dynamic light conditions and environmental stress (Pearcy et al. 2004). Thus, each leaf has a rather unique combination of trait values, all contributing to the canopy (Niinemets 2007). Integrating scales from single leaves to canopies is important, e.g., to quantitatively assess and predict carbon fluxes. Limited access to the leaves of many tree canopies and the limited throughput of currently available approaches make detailed long-term monitoring of selected leaves and branches within the canopy difficult.

Non-invasive probing of chlorophyll fluorescence has become a powerful method in plant biology over the last few decades (Papageorgiou and Govindjee 2005). There are several active methods to measure chlorophyll fluorescence, from a detailed analysis of polyphasic fluorescence rise after illumination (Strasser et al. 1995) to the application of saturating pulses to modulate chlorophyll fluorescence and derive photosynthetic parameters (Schreiber 1986). These approaches rely on an active modification of the light environment of the object of study. Use of these approaches outside of the laboratory is limited to individual leaves in accessible parts of the canopies. Passive fluorescence methods monitor sun-induced chlorophyll fluorescence, which gives information on photosynthetic processes over large vegetated areas (Malenovsky et al. 2009, Damm et al. 2010) but provides limited information on photosynthetic mechanisms.

The laser-induced fluorescence transient (LIFT) approach was developed to bridge the gap between laboratory and field measurements, and can be used in situ for long-term and automated monitoring of selected leaves. The approach has been cross-compared with commercial pulse amplitude-modulated (PAM) and gas exchange measurements (Ananyev et al. 2005, Kolber et al. 2005, Pieruschka et al. 2010) and has been successfully applied in a range of studies to detect the spatial variability of light-use efficiency (LUE) (Rascher and Pieruschka 2008, Nichol et al. 2012) and also the impact of cold stress on photosynthesis (Pieruschka et al. 2010). The widely used PAM fluorometers (Schreiber 1986) use high-intensity saturating pulses, more than two- to threefold greater than leaves experience in nature, in order to induce a maximum level of fluorescence. This maximum fluorescence level enables, in relation to a minimum fluorescence level, the calculation of quantum yield. The LIFT technique applies a sequence of sub-saturating excitation pulses at microsecond intervals to manipulate the light environment on the leaf surface to induce a fluorescence transient. This fluorescence transient is fitted to extrapolate a maximum fluorescence level (Kolber et al. 2005), which is then interpreted in analogy to the PAM approach. In contrast to the PAM approach, LIFT enables measurement from a distance of up to 50 m and may provide a unique opportunity to observe remotely inaccessible vegetation canopies.

In this study, we perform a long-term experiment that is designed to monitor the daily and seasonal dynamics of photosynthesis. The aim of this study is to investigate the acclimation and dynamics of photosynthetic efficiency of tree species caused by seasonal variations in environmental conditions. In particular, we evaluate the performance of LIFT within the tree canopies throughout a season by assessing it against the standard PAM approach.

Materials and methods

Experimental sites and tree species

The experiments were performed with four different tree species at two experimental sites located in California, USA, and Germany. At the Carnegie Institution for Science in Stanford, CA, USA (37°25′N, 122°9′W), photosynthetic efficiency of the evergreen oak *Quercus agrifolia* Née was measured between the end of February 2009 and mid-March 2009. The tree was ~15 m high and 30 years old. The second part of the experiment was performed with three different species, *Tilia cordata* L., *Quercus petraea* L. and *Pinus sylvestris* L., at the Forschungszentrum Jülich in Germany (50°55′N, 6°21′E) between July 2009 and January 2010. All experimental trees reached a height of ~15–20 m and were 20–30 years old.

Field experiments

In all field experiments, photosynthetic efficiency was monitored by the continuously measuring LIFT device and with commercial PAM fluorometers, specifically designed for long-term field experiments (monitoring PAMs, Porcar-Castell et al. 2008*b*). The selected leaves within tree canopies were clamped in the leaf clips of the three available monitoring PAMs under an angle mimicking approximately the natural position of the leaf. The monitoring PAMs were placed at the uppermost accessible part of the canopy, ~15 m above ground. Every 10 min a saturating pulse was applied and maximal (F_{mPAM}) and steady-state fluorescence prior to the flash (F_{PAM}) were measured. Every 1–2 weeks a new leaf was clamped into the leaf clip of the monitoring PAMs to reduce any potential photoinhibition of long-term application of saturating pulses. The quantum yield of Photosystem II (PSII) of light-adapted leaves ($\Delta F/F'_{mPAM}$) was calculated as follows: $\Delta F/F'_{mPAM} = (F'_{mPAM} - F_{PAM})/F'_{mPAM}$, with F'_{mPAM} as maximum fluorescence and F'_{PAM} minimum fluorescence of light-adapted leaves. Electron transport rates (ETR) for the PAM measurements were assessed by $ETR_{PAM} = \Delta F/F'_{mPAM} \times PPFD_{PAM} \times 0.84 \times 0.5$, with 0.84 being an estimate of absorbed photosynthetically active photon flux density ($PPFD_{PAM}$) measured by the sensor of the monitoring PAMs and 0.5 accounting for the partitioning of light absorption between PSI and PSII (Genty et al. 1989). The quantum yield of PSII of dark-adapted leaves (F_v/F_{mPAM}) was calculated as $F_v/F_{mPAM} = (F_{mPAM} - F_{oPAM})/F_{mPAM}$, with F_{oPAM} being the minimum fluorescence and F_{mPAM} the maximum fluorescence of dark-adapted leaves (Maxwell and Johnson 2000). F_v/F_{mPAM} was calculated as an average of values acquired 1–2 h before sunrise.

The LIFT system was set up ~40–50 m away from the canopies, measuring four areas of interest (AoIs) using an excitation beam of 10 cm diameter projected in the vicinity of each of the monitoring PAMs. LIFT measurements were performed continuously with every new data point obtained at intervals of 5–6 min. The LIFT approach used a laser-based excitation source (665 nm), operating in pulse mode with variable duty cycle. At the high duty cycle, 100 flashlets of 5 µs duration and at 5 µs intervals were used to manipulate the photosynthetic activity within PSII reaction centers. At the low duty cycle, 40 flashlets lasting 5 µs were applied with exponentially increasing intervals to observe the kinetics of photosynthetic electron transport. During the measurements, 50 of the so-called pulse trains were measured and averaged to increase the signal-to-noise ratio. The maximum (F_{mLIFT}) and steady-state fluorescence was calculated by numerically fitting the fluorescence transient to a theoretical model that describes the relationship between fluorescence and photosynthetic light conversion (Kolber et al. 1998, 2005). The quantum efficiency of dark- (F_v/F_{mLIFT}) and light-adapted leaves ($\Delta F/F_{mLIFT}$) was calculated in analogy to the PAM approach and multiplied with an empirical factor $\varphi = 1.628$. This factor accounts for the difference between the PAM and LIFT approaches derived from the differences in the linear correlation of these two approaches with gas exchange measurements (for details see Pieruschka et al. 2010). ETR_{LIFT} was calculated in analogy to the PAM approach. $PPFD_{LIFT}$ required for the calculation of ETR_{LIFT} was taken from meteorological stations at each of the experimental sites.

Stanford site

During the experiment at the Carnegie Institution in Stanford, three monitoring PAMs were placed at the top and in the middle part of the sun-exposed canopy of *Q. agrifolia* and at a shady spot at the bottom of this tree. The LIFT was placed on a 5-m-high platform at the Carnegie building and scanned the top, middle and bottom part of the southern-exposed canopy from a distance of ~40–50 m. Meteorological data and shortwave radiation (300–2500 nm) were measured at a meteorological station at the Carnegie Institution with a pyranometer every 30 min. The radiation was converted into $PPFD_{LIFT}$ by multiplying the obtained radiation (W m^{-2}) with the calibration factor equal to 4.6 (Ting and Giacomelli 1987).

Jülich site

Photosynthetic efficiency was measured within the canopies of the three tree species, *T. cordata, Q. petraea* and *P. sylvestris*, from mid-July 2009 to the end of September 2009. The monitoring PAMs were located in the upper part of the canopy of each of these trees. The LIFT was placed on the roof of a 25-m-high building, monitoring the southwestern-exposed side of the deciduous trees and the western-exposed part of the pine tree crown from a distance of 40–50 m. At the Stanford site, four AoIs around each of the monitoring PAMs were assessed with LIFT. At the end of September, the deciduous trees started to turn yellow and PAM fluorescence was measured only in the pine canopy until the experiment ended at the end of January. In Jülich, the meteorological data were collected at the meteorological station and radiation (300–3000 nm) was measured every minute by a pyranometer (Type CM7, Kipp & Zonen, Delft, The Netherlands) on the roof of the same building where the LIFT was installed. $PPFD_{LIFT}$ was calculated in analogy to the calculation at the Stanford site.

Data analysis

The dependence of the monitoring-PAM fluorometer on temperature was tested with a fluorescence standard at temperatures ranging from –15 to 25 °C. A linear dependence between fluorescence measurements and temperature was found and the fluorescence values obtained during the experiments were, therefore, corrected accordingly. $PPFD_{LIFT}$ data, acquired with previously described pyranometers, were used to calculate ETR_{LIFT} when taken simultaneously with LIFT measurements. For the rest, the measured $PPFD_{LIFT}$ data were interpolated to derive irradiance data required for ETR_{LIFT}.

For calculation of the daily PPFD ($PPFD_{day,LIFT}$) and ETR ($ETR_{day,LIFT}$), the diurnal curves were generated by linearly fitting data points between two subsequent time intervals, which were then integrated over the entire day. We acknowledge that this approach may introduce some error in calculating ETR, especially during fluctuating light conditions. Mean daily LUE, which represents the daily average operating efficiency of PSII, was calculated from the relation $LUE = ETR_{day}/PPFD_{day}$ for LIFT and PAM. Statistical analysis was performed with Student's *t*-test at a significance level of 0.05 and with a null hypothesis that there is no significant difference between the treatments. Maximum electron transport rates ($ETR_{MAX,PAM}$) were calculated with the PAM approach by fitting the ETR vs PPFD relation with a two-parameter exponential curve (cf. Rascher et al. 2000). The *F*-test was used to analyze the differences between different slopes of the ETR and PPFD relations of different species, and between LIFT and PAM. The analysis was performed with the SigmaPlot software (Systat, Inc., Erkrath, Germany, Version 11).

The relationships between ETR, PPFD and $\Delta F/F_m'$ were analyzed by using a scaling slope analysis (Renton and Poorter 2011). These values, corresponding to the multiplicative relation $ETR = PPFD \times \Delta F/F_m' \times \kappa$ (where $\kappa = 0.5 \times 0.84$, the PSII/PSI light partitioning and leaf absorbance constants), were logarithmically transformed, and ln(ETR) was plotted vs ln(PPFD) and $\ln(\Delta F/F_m')$. The slopes of these relations indicate the relative contribution of PPFD or $\Delta F/F_m'$ to the variability of ETR. To test the dependence of ETR on PPFD and temperature, the parameters were linearly fitted and the regression coefficient of determination (R^2) was computed.

Results

The field experiment at the Stanford site was performed during spring with average temperatures of ~12 °C and $PPFD_{day,LIFT}$ ranging between 15 and 25 mol m^{-2} day^{-1} (Figure 1a). At the Jülich site, the summer temperatures averaged 20 °C and decreased gradually during the fall, with two distinctive minima in the winter. The corresponding $PPFD_{day,LIFT}$ showed some considerable variation during the summer with values between 30 and 55 mol m^{-2} day^{-1}, and decreased gradually in the fall to a value of ~5 mol m^{-2} day^{-1} (Figure 1a). During the experiment at the Jülich site, precipitation often reached >20 mm per week throughout the experimental period so that drought stress is very unlikely (Figure 1b). The wind conditions during the experiment were calm, with maxima of up to 10 m s^{-1} in the fall and winter (Figure 1b), which still necessitated frequent readjustment of the monitoring PAMs within the tree canopies because leaves were ripped off the leaf clip. During the experimental period, photosynthetic efficiency followed changes in $PPFD_{day,LIFT}$ with some species-specific differences, resulting in highest values for *T. cordata*, intermediate values for *Q. petraea* and lowest values for *P. sylvestris*. The $ETR_{day,LIFT}$ of *P. sylvestris* reached a minimum of ~0.5 mol m^{-2} day^{-1} in November and remained low until the end of the experiment (Figure 1c).

A representative day course of PPFD and fluorescence parameters for the tree species at the Jülich site is shown in Figure 2. $PPFD_{LIFT}$ corresponded to the irradiance outside of the canopy, while $PPFD_{PAM}$ represented the incident light environment on leaves clipped in the monitoring PAMs (Figure 2a–c). The resulting fluorescence parameters obtained by LIFT indicate rather small differences between F_{LIFT} and F'_{mLIFT} in the canopy of *P. sylvestris*, resulting in a not very distinct $\Delta F\ F'^{-1}_{m\,LIFT}$ diurnal pattern (Figure 2d). The difference between F_{LIFT} and F'_{mLIFT} obtained for *T. cordata* (Figure 2e) and *Q. petraea* (Figure 2f) resulted in a diurnal pattern of $\Delta F\ F'^{-1}_{m\,LIFT}$ with minima in the afternoon. Nonphotochemical quenching measured by LIFT (NPQ_{LIFT}) in particular for *T. cordata* and *Q. petraea* (Figure 2n and o) also showed a maximum in the afternoon. However, ETR_{LIFT} (Figure 2g–i) followed the diurnal $PPFD_{LIFT}$ course (Figure 2a–c). The complementary fluorescence parameters recorded by the monitoring PAM, F_{PAM}, F'_{mPAM} and $\Delta F\ F'^{-1}_{m\,LIFT}$ (Figure 2g–i) as well as ETR_{PAM} (Figure 2j–l) and NPQ_{PAM} (Figure 2m–o), were largely affected by the fluctuating incident light on the measured leaves (Figure 2a–c).

To compare the two different approaches, ETR_{LIFT} and ETR_{PAM} were plotted vs the respective PPFD and linearly fitted (Figure 3). The ETR_{PAM} values of a smaller PPFD dynamic range fit within the envelope of larger ETR_{LIFT} scatterplots. The linear slopes for the PAM approach were rather similar to those

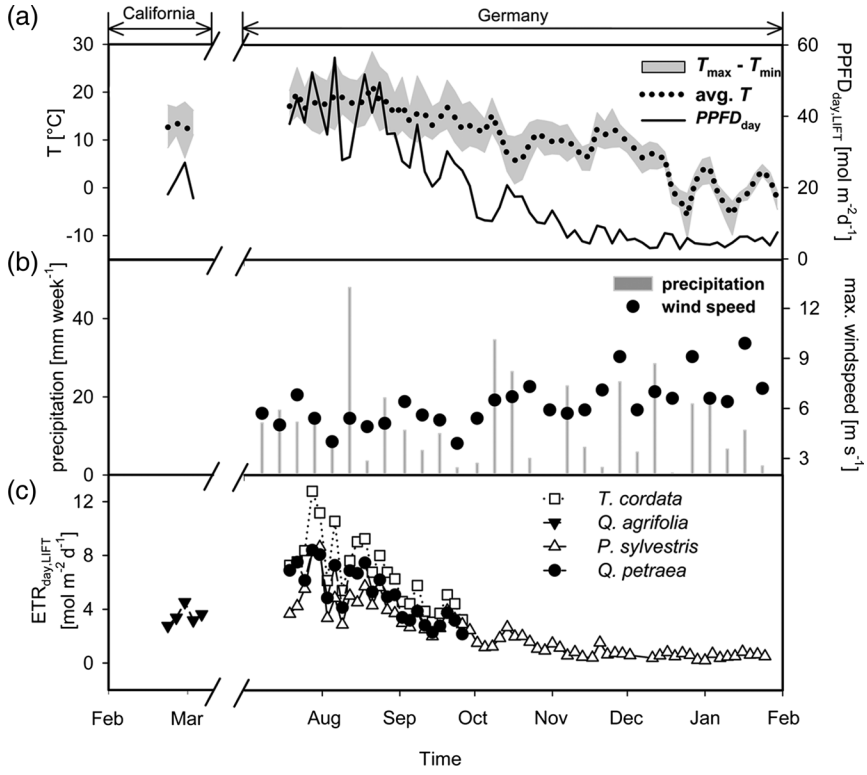

Figure 1. (a) Photosynthetically active photon flux density (solid line), temperature range (gray area) and the average temperature (*T*) (dotted line) recorded during the experimental period; (b) precipitation (gray bars) and wind speed (closed symbols) during the experiment; (c) seasonal dynamics of ETR recorded by LIFT for the investigated tree species. Stanford site: *Q. agrifolia*; Jülich site: *Q. petraea*, *T. cordata* and *P. sylvestris*.

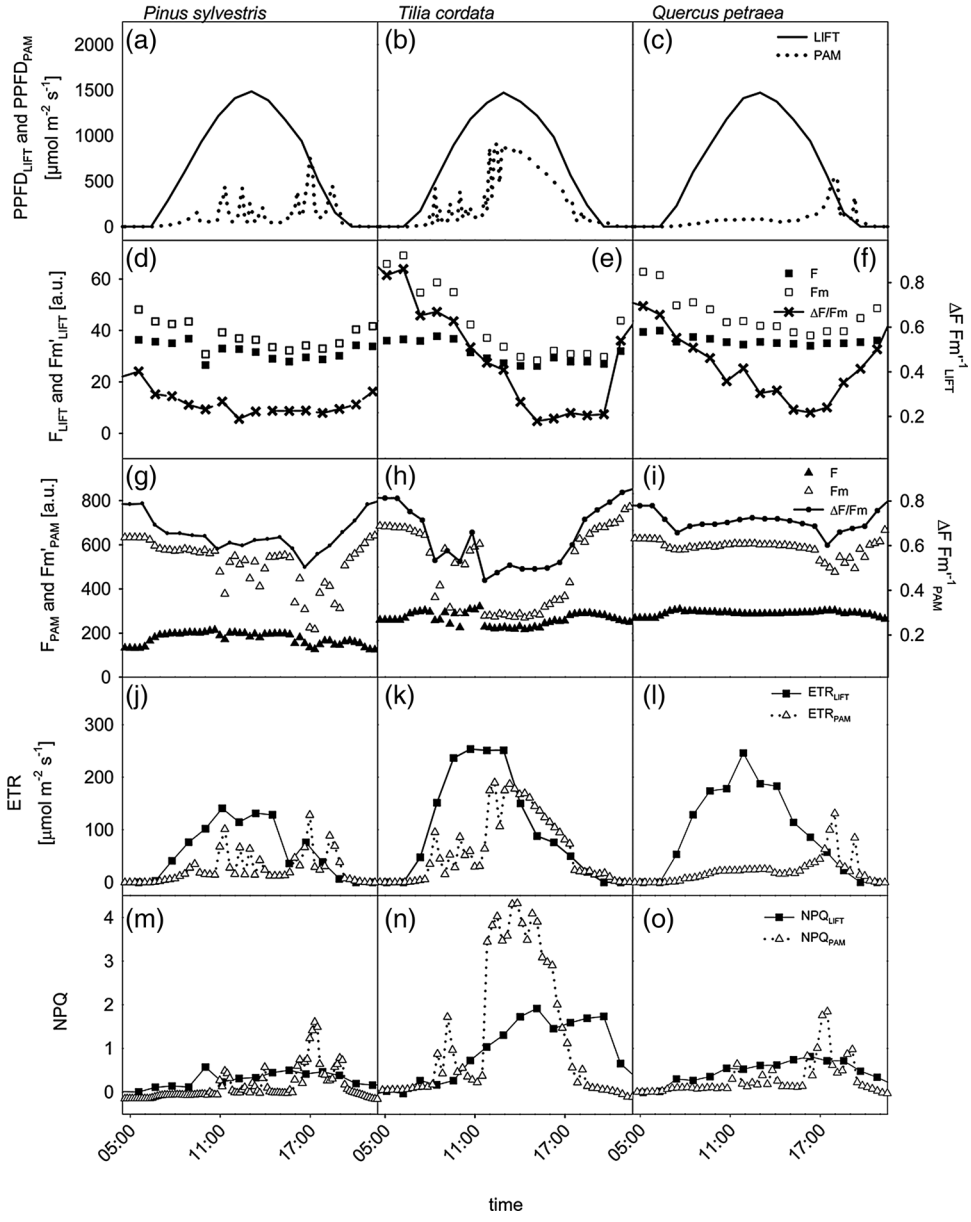

Figure 2. (a)–(c) Diurnal course of the PPFD measured outside the canopy ($PPFD_{LIFT}$) as a function of local time and $PPFD_{PAM}$ measured by the sensors of the monitoring PAMs; (d)–(f) the corresponding fluorescence parameters measured by LIFT, minimum fluorescence (F_{LIFT}), maximum fluorescence (F'_{mPAM}) and quantum efficiency ($\Delta F\ F'^{-1}_{mLIFT}$); (g)–(i) fluorescence parameters measured by PAM, minimum fluorescence (F_{PAM}), maximum fluorescence ($F'_{m,PAM}$) and quantum efficiency ($\Delta F\ F'^{-1}_{mPAM}$); (j–l) ETR measured by LIFT (ETR_{LIFT}) and PAM (ETR_{PAM}) and (m–o) non-photochemical quenching measured by LIFT (NPQ_{LIFT}) and PAM (NPQ_{PAM}).

of the LIFT approach for *T. cordata* (Figure 3a), *Q. petraea* (Figure 3b) and *Q. agrifolia* (Figure 3c), but steeper in the case of *P. sylvestris* (Figure 3d). The *F*-test, however, revealed no statistically significant difference between LIFT and PAM measurements of all the different species (data not shown).

The quantum yield of dark-adapted leaves (F_v/F_m) indicated a seasonal decrease towards the fall and winter. For both *T. cordata* and *Q. petraea*, the F_v/F_m curves based on LIFT and PAM showed good agreement (Figure 4a and b). In mid-August, the leaves on the outer part of the canopy of *Q. petraea* started to turn yellow. The decrease in leaf chlorophyll content is demonstrated as a steeper decrease in LIFT quantum yield measurements, but it was not detected by the PAM approach (Figure 4b). LIFT F_v/F_m values of the evergreen species *Q. agrifolia* and *P. sylvestris* were mainly between 0.3 and 0.6, i.e., substantially lower than the PAM-based F_v/F_m (Figure 4c and d). In particular, F_v/F_m measurements of *P. sylvestris* during the winter may indicate foliage adaptations to fall and winter conditions (Figure 4d). To empirically investigate the effect of leaf size and geometrical arrangement on the fluorescence signal, the ratio of F_v/F_m recorded by LIFT and PAM for the studied species and a number of species from previous

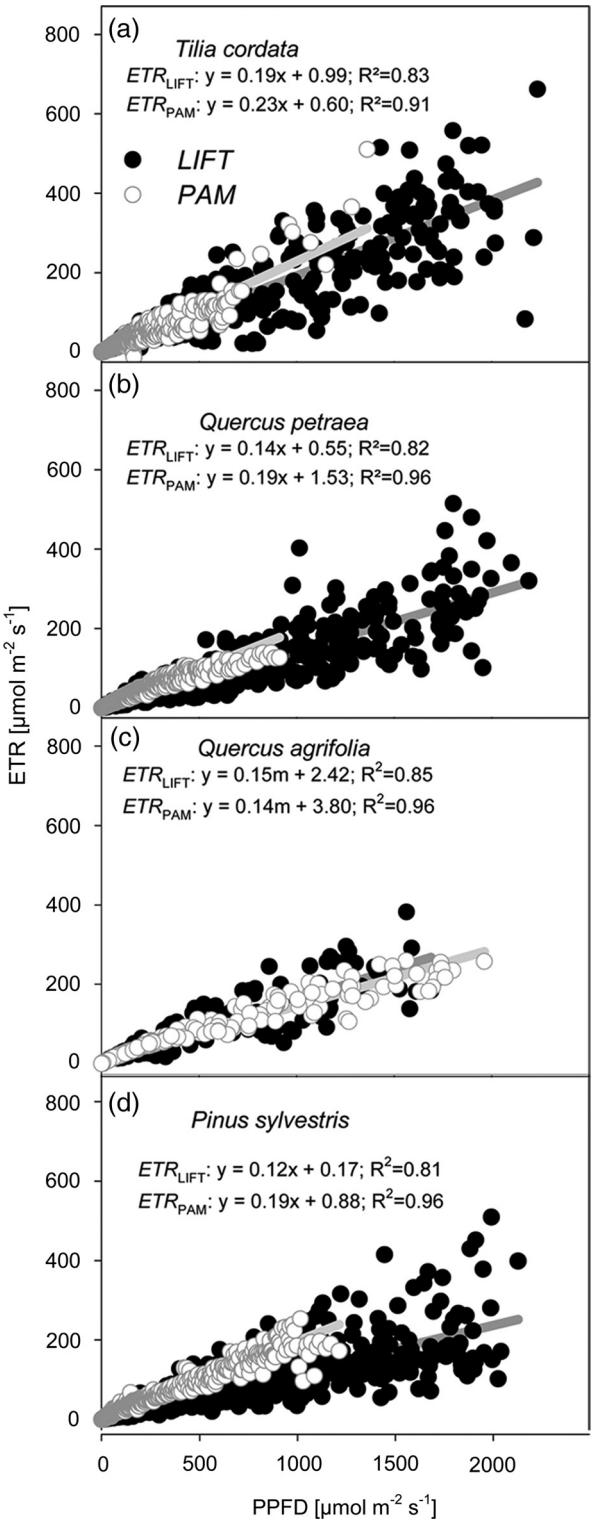

Figure 3. Relation of ETR recorded by PAM (ETR_{PAM}) and LIFT (ETR_{LIFT}) and PPFD obtained for (a) *T. cordata*, (b) *Q. petraea*, (c) *Q. agrifolia* and (d) *P. sylvestris*.

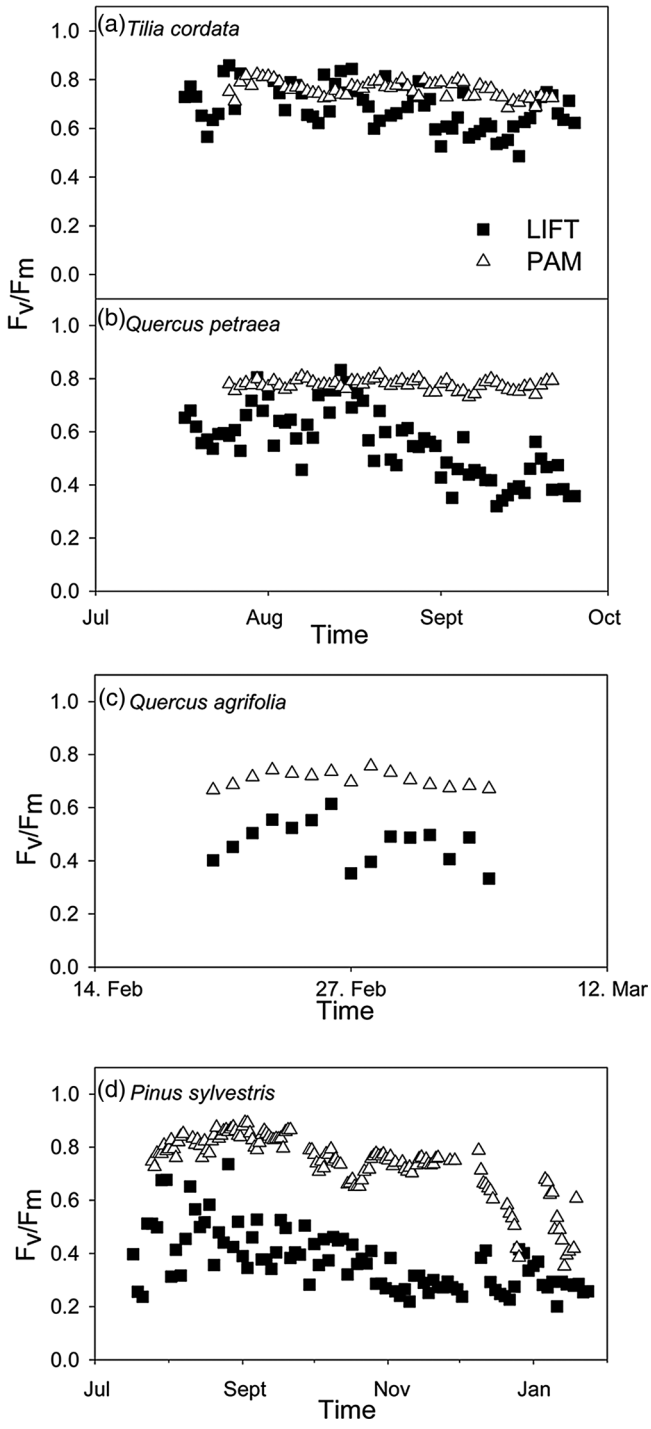

Figure 4. Predawn quantum yield (F_v/F_m) recorded with LIFT (closed squares) and PAM (open symbols) for (a) *T. cordata*, (b) *Q. petraea*, (c) *Q. agrifolia* and (d) *P. sylvestris*.

studies was plotted vs the potential maximal leaf area that can be probed by the collimated LIFT beam normalized by the size of this beam. For plant species with rather large leaves, the F_v/F_m values recorded by LIFT were similar to the PAM values, but with decreasing leaf area and increasing canopy structural complexity the LIFT signal appeared to be affected by the anisotropy of the leaf clumps (Figure 5).

Detailed information on the photosynthetic status of *Q. agrifolia* was derived by comparing different parts of the canopy of *Q. agrifolia* at the Stanford site (Table 1). Here, the

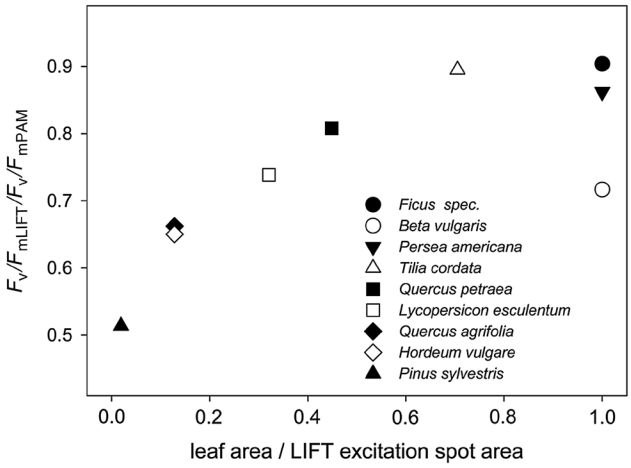

Figure 5. Relation of the ratio of F_v/F_{mLIFT} vs F_v/F_{mPAM} on leaf size described as the ratio of the potentially maximal leaf area that can be measured by a LIFT beam with a diameter of 10 cm to the size of the LIFT beam. Data were taken from *T. cordata*, *Q. petraea*, *Q. agrifolia* and *P. sylvestris* (current study), *Persea americana* Mill., *Lycopersicon esculentum* Mill. (Pieruschka et al. 2010), *Beta vulgaris* L., *Hordeum vulgare* L. and *Ficus* sp. L. (unpublished data). Leaf size was taken from own measurements.

Table 1. Photosynthetic parameters of *Q. agrifolia* measured in the early spring within different canopy layers (top, middle, bottom). The letters indicate statistically significant differences between different canopy layers.

Parameter	Top	Middle	Bottom	*n*
F_v/F_{mLIFT}	0.48 ± 0.08^a	0.49 ± 0.07^a	0.61 ± 0.08^b	15
F_v/F_{mPAM}	0.71 ± 0.03^a	0.65 ± 0.03^b	0.68 ± 0.04^{ab}	12
LUE_{LIFT}	0.14 ± 0.04^a	0.13 ± 0.04^a	0.18 ± 0.04^b	15
LUE_{PAM}	0.17 ± 0.03^a	0.13 ± 0.03^b	0.20 ± 0.03^c	12
$ETR_{MAX,PAM}$ ($\mu mol\ m^{-2}\ s^{-1}$)	364 ± 4	153 ± 1	62 ± 1	1[1]

[1]$ETR_{MAX,PAM}$ was obtained by plotting data from the entire measurement period.

F_v/F_{mLIFT} obtained at the top and the middle of the canopy was substantially lower than at the bottom, whereas the F_v/F_{mPAM} showed rather small differences between the canopy parts. Light-use efficiency, derived from PAM and LIFT measurements, was lowest in the middle part of the canopy, while the highest values were observed at the shaded bottom of the canopy. Maximum electron transport capacity (ETR_{MAX}) was largest at the top of the canopy and lowest at the bottom (Table 1).

The seasonal dynamics of mean daily LUE_{LIFT}, ranging between 0.1 and 0.25, showed a similar variation to PAM measurements, but with distinct differences between the species (Figure 6). The heterogeneous needle-leaf canopy of *P. sylvestris* showed lower LUE_{LIFT} than the broad-leaf canopy of *Q. petraea* and the compact canopy of *T. cordata*, which revealed, in agreement with ETR_{LIFT} measurements (Figure 1), the highest values. LUE_{PAM} ranged between 0.15 and 0.3, with rather small differences between the species. Another characteristic difference occurred for *P. sylvestris*, where LUE_{PAM} varied considerably, mainly due to the freezing events in the winter, while LUE_{LIFT} was rather stable throughout the year, varying only slightly in August and from the end of December to the beginning of January (Figure 6a and b). The scaling slope analysis was performed to analyze the impact of PPFD and LUE ($\Delta F/F_m'$) on ETR. PPFD and $\Delta F/F_m'$ recorded for each day throughout the experimental period were logarithmically converted and plotted vs the logarithmically converted ETR (Figure 7a and b). $PPFD_{LIFT}$ with values ranging between 0.79 and 1.15 for LIFT and between 1.00 and 1.22 for $PPFD_{PAM}$, respectively (Figure 7a), had a substantially larger influence on ETR than $\Delta F/F_m'$. The scaling slope values for $\Delta F/F_m'$ vs ETR ranged between −0.15 and 0.25 for LIFT and between −0.17 and 0.00 for PAM, respectively (Figure 7c and d). To

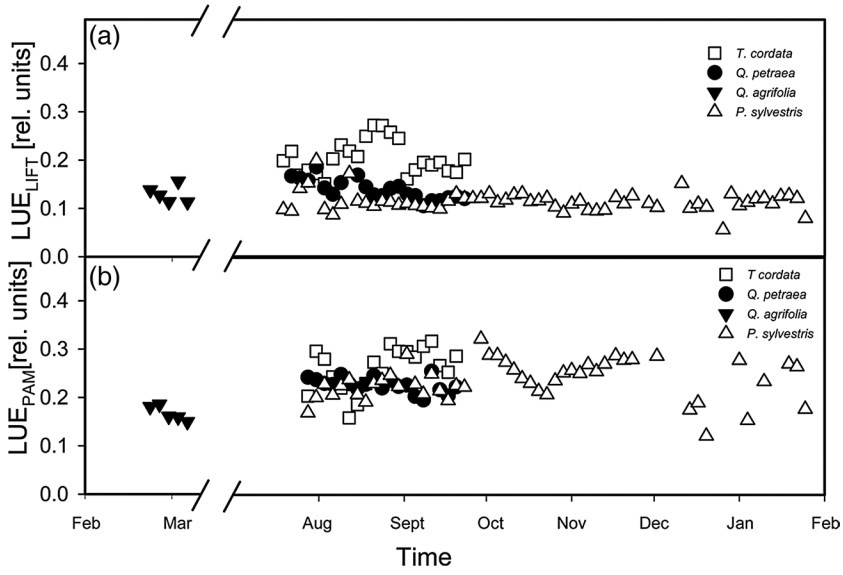

Figure 6. Light-use efficiency (LUE) as the average operating photochemical efficiency recorded over the experimental period by (a) LIFT and (b) PAM. Stanford site: *Q. agrifolia*; Jülich site: *Q. petraea*, *T. cordata* and *P. sylvestris*.

test the impact of the temperature on ETR, the diurnal course of temperature and ETR were plotted against each other and the resulting R^2 of this relationship throughout the experimental period is shown in Figure 7e and f. In all cases R^2 was not >0.55, which indicates a weak correlation between ETR and temperature. The analysis of the seasonal data also supported the scaling slope analysis where ETR was mostly affected by PPFD but not temperature (Table 2). However, during the transition from fall to winter the LIFT parameters, F'_{mLIFT} and F_{LIFT}, were observed to be hardly affected by the decreasing temperature (Figure 8a and b) while the PAM-based maximum fluorescence F'_{mPAM} decreased substantially when the minimum temperature approached 0 °C (Figure 8d) and F'_{oPAM} was less affected (Figure 8c). The light-adapted fluorescence parameters, F'_m and F, showed a similar dependence on temperature (data not shown).

Discussion

Photosynthetic performance was monitored successfully in four tree species at two spatial scales over both single

Table 2. Regression coefficient (R^2) for the seasonal correlation between ETR and PPFD or temperature obtained with LIFT or PAM.

Plant species	LIFT/PAM	R^2	
		ETR vs PAR	ETR vs *T*
T. cordata	LIFT	0.7895	0.1757
	PAM	0.9347	0.1384
Q. petraea	LIFT	0.7430	0.2153
	PAM	0.9541	0.2080
Q. agrifolia	LIFT	0.9130	0.3300
	PAM	0.9872	0.0162
P. sylvestris	LIFT	0.8349	0.4080
	PAM	0.9096	0.0057

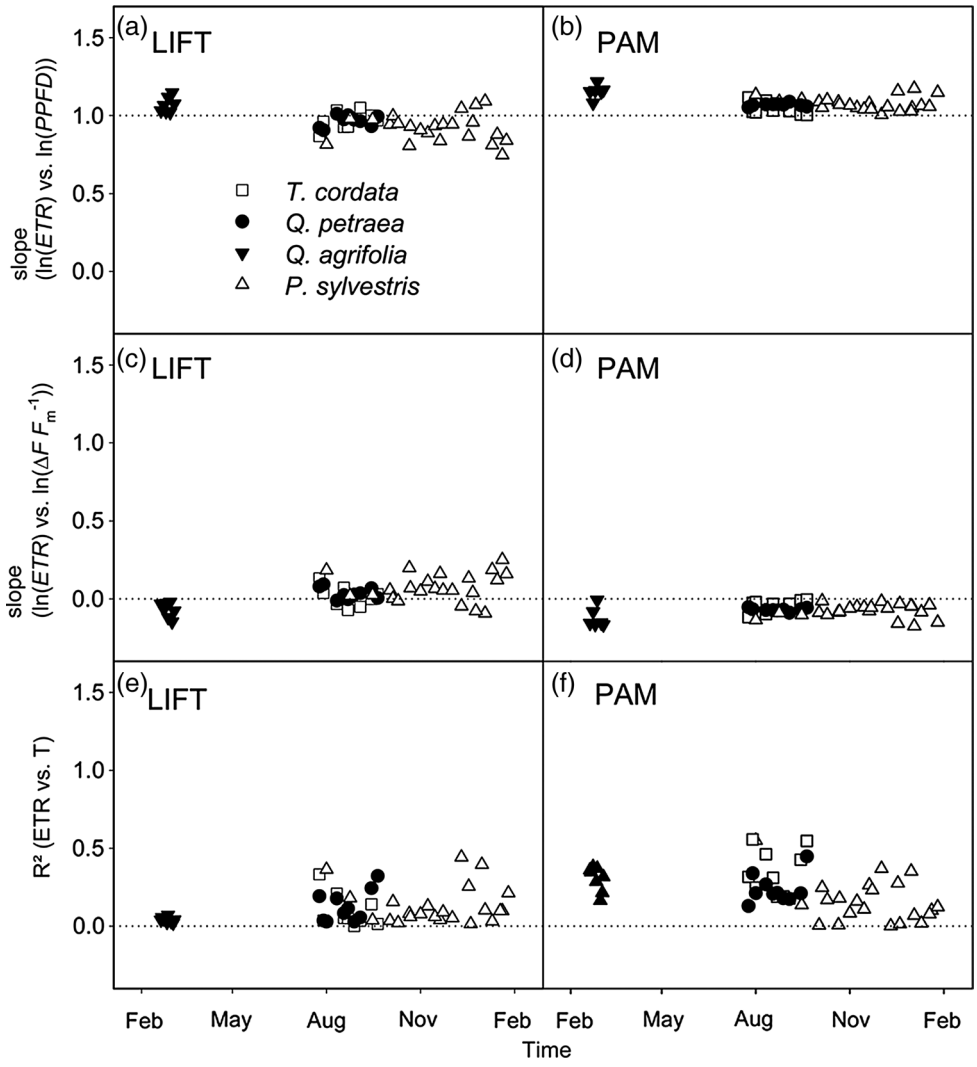

Figure 7. (a) and (b) Slope of the relation ln(ETR) vs ln(PPFD) with (a) LIFT- and (b) PAM-based measurements. (c) and (d) Slope of the relation ln(ETR) vs ln($\Delta F\ F_m'^{-1}$) with (c) LIFT- and (d) PAM-based measurements. (e and f) Regression coefficient for the ratio of ETR vs temperature for *T. cordata*, *Q. petraea*, *Q. agrifolia* and *P. sylvestris*.

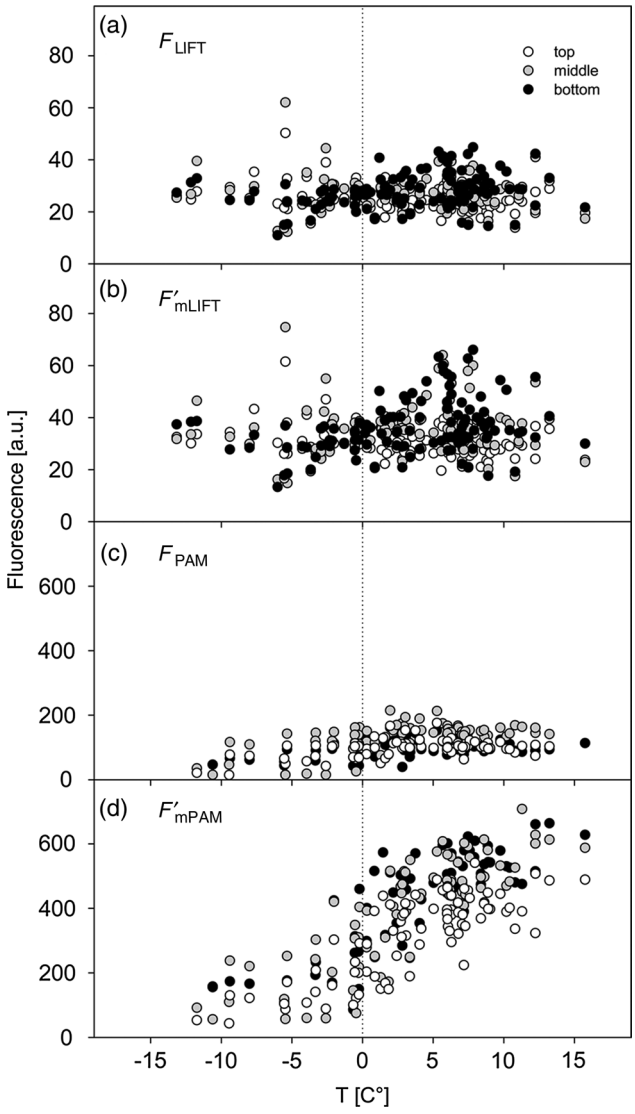

Figure 8. Predawn steady-state fluorescence (F) and maximum fluorescence (F_m) at the top (open symbols), middle (gray symbols) and bottom part (black symbols) of the *P. sylvestris* canopy as a function of minimum air temperature (T) obtained during the transition from fall to winter (October—January) with LIFT (a and b) and with PAM (c and d).

days (Figure 2) and extended time periods such as seasons (Figure 1). The LIFT approach showed photosynthetic parameters at a novel spatial scale, leaf and branches determined at 40–50 m distance, which was confirmed by simultaneous measurements of PAM measurements at leaf level. The LIFT instrument compares reasonably well with the PAM leaf measurements under field conditions in terms of ETR for *T. cordata*, *Q. petraea* and *Q. agrifolia* (Figure 3), i.e., broadleaf canopies. Noticeable differences, however, occurred between ETR_{PAM} and ETR_{LIFT} (Figure 3) as well as for F_v/F_{mLIFT} and F_v/F_{mPAM} for small leaves, in particular for the conifer canopy of *P. sylvestris* (Figures 5 and 3d). As observed in a previous study (Pieruschka et al. 2010), LIFT measurements conducted on smaller leaves, in particular narrow grass leaves, resulted in larger signal variability than measurements of large broad leaves. The excitation beam of LIFT (~10 cm in diameter) is in many cases larger than the measured leaves (Figure 5) and thus the signal recorded with LIFT integrates information of several foliage layers within the canopy. Therefore, the detected fluorescence originating from the layers exposed to different light environments may differ from pulse train to pulse train. Secondly, depending on the specific canopy structure and leaf biochemical/optical properties (i.e., angle distribution, clumping, chlorophyll and water content, etc.), the fluorescence signal is scattered and re-absorbed by the foliage elements as any other photon flux. As demonstrated by Knyazikhin et al. (2013), these optical processes are known to be stronger in canopies of small narrow leaves with a higher photon recollision probability. Finally, as the foliage is moving in the wind, the fluorescence emitted from a small-leaf canopy is scattered and re-absorbed slightly differently each time it is measured by the LIFT detector. Therefore, a narrow-leaf canopy, as for instance the pine tree, resulted in an underestimation of ETR_{LIFT} vs ETR_{PAM} or F_v/F_{mLIFT} vs F_v/F_{mPAM}, which is not obvious in the case of *T. cordata*, where the relatively large leaves of the outer canopy, arranged like 'roof tiles', minimize the confounding canopy structural effects. Additionally, the dependence of the LIFT fluorescence signal on leaf size and canopy structure may also be affected by a flat measurement angle of the LIFT beam relative to the canopy, which was identified in a previous study with *Beta vulgaris* L. (data not shown) as the cause of an underestimation of the F_v/F'_{mLIFT} signals (Figure 5).

The $PPFD_{PAM}$ on the leaves mounted in the monitoring PAM differed from the $PPFD_{LIFT}$ on the outer layer of the canopy (Figure 3). In the case of *Q. agrifolia*, the monitoring PAM was attached to a dead branch sticking out of the canopy, which ensured that a leaf of the outer layer was clipped and hardly shaded. For the other species it appeared more difficult to access leaves of thin branches moving in the wind without self-shading, which resulted in substantially lower $PPFD_{PAM}$ (Figure 3). The monitoring PAMs tracked the actual incident light environment on the leaf surface, which enabled an accurate assessment of diurnal (Figure 2) and seasonal (Figure 1) fluorescence parameters of the selected leaves, which mostly followed the variation in light. However, long-term application of saturating pulses by the PAM may cause substantial photoinhibition (Shen et al. 1996, Apostol et al. 2001). This applies especially for needle leaves, which when forced into the PAM leaf clip may be strongly affected by saturating pulses applied to the 'side' of the needles, while the natural light environment within needle clumps is rather of lower intensity and diffuse in nature. We accounted for that by replacing and measuring new leaves every 1–2 weeks. The incident light intensity on the leaves probed with LIFT could not be measured directly and was assumed to be similar to $PPFD_{LIFT}$, i.e. the intensity outside

the tree canopy measured with horizontally oriented sensors. The diurnal pattern of the fluorescence parameters showed a difference between the maximum $PPFD_{LIFT}$ (Figure 2b and c) and the minimum of F'_{mLIFT} and $\Delta F\ F'^{-1}_{m\ LIFT}$ as well as the maximum for NPQ_{LIFT} for *T. cordata* (Figure 2e and n) and *Q. petraea* (Figure 2f and o), which occurred with some delay after the solar noon. This can be explained by physiological processes related to mid-day depression, which is well known to induce stomatal closure in the afternoon and affect the fluorescence parameters (Medrano et al. 2002). Additionally, $PPFD_{LIFT}$ was recorded with a horizontal sensor whereas the leaves of the outer layer of the canopies of *T. cordata* and *Q. petraea* have an inclination resulting in perpendicular light exposure and thus the highest light exposure in the afternoon affecting fluorescence. Considering all these differences, it is expected that LIFT and PAM measurements and derived photosynthetic parameters are not fully comparable, even though they compare relatively well in broadleaf cases (Figures 3 and 4).

During the cold acclimation of *P. sylvestris*, low temperatures induced a decrease and higher variability of LUE_{PAM}, whereas the LUE_{LIFT} measurements were almost unaffected. Figure 8 shows that LIFT-measured steady state (F) and maximum fluorescence (F_m) were not much affected by temperatures <0 °C during the winter period. Contrary to this, the PAM measurements, in particular F'_{mPAM}, decreased substantially when the minimum air temperature approached 0 °C, as previously observed by Soukupová et al. (2008). The differences may be related to the above-described structural effects of a narrow-leaf canopy that alternate the leaf chlorophyll fluorescence signals. The confounding canopy effects seem to cause a lower variability of LIFT fluorescence and derived LUE measurements. It is foreseen that sensitivity analyses conducted using a three-dimensional canopy radiative transfer model, similar to the SCOPE model (van der Tol et al. 2009) with a capacity to simulate the forest canopy chlorophyll fluorescence, could verify and help to understand the behavior of LIFT measurements illustrated in Figures 6 and 8. Additionally, the fluorescence signal of the PAM apparatus is measured with a long-pass filter for wavelengths >650 nm (E.E. Pfündel, personal communication). The LIFT apparatus uses a narrow 10-nm band-pass filter centered at 690 nm. Thus, while the PAM apparatus detects the fluorescence emitted by PSII and PSI, the LIFT apparatus detects only PSII fluorescence. Under normal conditions, the contribution of PSI to the fluorescence signal is rather low, with relatively low variability. However, it was shown that chilling stress combined with a high-intensity light is able to inhibit PSI (Kudoh and Sonoike 2002, Zhang and Scheller 2004). Depending on plant species, different levels of damage of PSII, PSI or both may occur under low temperature and irradiation stress (Scheller and Haldrup 2005). While an enhanced activity of PSI induced by cyclic electron transport is a well-known phenomenon (Huner et al. 1998, Ivanov et al. 2001), PSI photoinhibition in *P. sylvestris* needles under cold stress has never been reported. We acknowledge that attributing the differences between LIFT and PAM F_m to PSI activity is hypothetical, and further dedicated measurements are required to verify this hypothesis.

In agreement with the study by Wullschleger (1993), which demonstrated a lower photosynthetic capacity of evergreens when compared with deciduous trees, our ETR observation is higher for the deciduous trees *T. cordata* and *Q. petraea* than for the evergreens *P. sylvestris* and *Q. agrifolia* (Figure 1). To the best of our knowledge, the observed species were not exposed to any intensive biotic or abiotic stress (see Figure 1a and b), but were affected only by seasonal downregulation and upregulation processes. These processes, often observed in evergreens (Gilmore and Ball 2000, Öquist and Huner 2003, Ensminger et al. 2006, Porcar-Castell et al. 2008*a*, Porcar-Castell 2011), are associated with the balance between the energy absorbed through largely temperature-insensitive photochemical processes and the energy used for temperature-sensitive biochemical processes and growth. During the spring, the processes are reversed and photosynthesis is re-activated. In the fall and winter (*P. sylvestris*) and also in spring (*Q. agrifolia*), the LIFT-recorded F_v/F_m was lower than that in the summer (Figure 4c and d), which may be related to the seasonal downregulation and upregulation processes. The PAM-based measurements in Figure 3d, however, indicate that the ETR_{PAM} vs $PPFD_{PAM}$ relation for *P. sylvestris* is higher than the LIFT-based values, while being comparable to the measurements obtained for *Q. petraea* (Figure 3b). As discussed previously, we assume that structural features of tree canopies related to leaf size, angle and distribution confound the LIFT measurements and result in underestimation of the quantum yield of needle-leaf canopies.

Despite large differences in ETR_{LIFT} throughout the season (Figure 1), LUE (ETR/PPFD) showed rather small seasonal variations (Figure 6). LUE_{LIFT} varied more in the summer than in fall and winter (Figure 6a), whereas LUE_{PAM} varied substantially more with the onset of freezing temperatures (Figure 6b). We assume that freezing water on the leaves at night and its melting during the day as well as some snow events caused these LUE_{PAM} fluctuations observed in December and January (Figure 6). Using a scaling slope analysis (Renton and Poorter 2011), the relative influence of LUE (or the analogous $\Delta F/F'_m$) and PPFD on ETR can be derived for the daily variation over the entire measurement period (Figure 7). PPFD had the largest influence on ETR with scaling slope values close to one (Figure 7a and b), whereas the impact of $\Delta F/F'_m$ (actual measure of LUE) on ETR appeared to be low (Figure 7c and d). Regression analysis over the measurement period supports the strong impact of PPFD on ETR (Table 2). Regression of diurnal courses of ETR vs temperature (Figure 7e and f) and the seasonal course (Table 2) indicates a rather small impact of temperature. This result is in agreement with the previous study by Turner et al.

(2003), who found that LUE was not well correlated with temperature. Although our experiments were performed with trees not exposed to severe stress impacts, some substantial variation in LUE was still observed which may be related to increasing stress levels (Figure 6a and b, Figure 7c and d).

Light-use efficiency (and other key photosynthetic parameters) indicated differences between the sunny and shaded parts of the *Q. agrifolia* canopy (Table 1), with higher LUE in the shaded and lower LUE in the sunny canopy parts but higher electron transport capacity in the sunny part of the canopy. This phenomenon is usually related to light acclimation resulting in sun and shade leaves, with associated specific characteristics such as foliar pigment composition and concentration, leaf size and specific leaf area (Lichtenthaler et al. 1981, Hallik et al. 2012). Variation of PPFD over days had, however, almost no influence on LUE of the outer canopy layer ($R^2 < 0.05$, data not shown). The only exception was an increasing tendency of LUE with increasing PPFD in the conifer canopy during the fall ($R^2 = 0.15$), and LUE decrease with PPFD decrease in the evergreen *Q. agrifolia* during the spring ($R^2 = 0.19$). Acclimation of canopies to PPFD variation is very gradual and occurs rather slowly during the season, which results in almost unchanged LUE (Figure 6). Acclimation to fast fluctuating light conditions on a daily scale would most likely require more resources than the additional gain by enhanced photosynthesis (Field 1983, Niinemets et al. 2004). In our experiment, we focused on the outer layers of the canopy which, with the exception of *Q. agrifolia,* showed very typical sun and shade photosynthetic traits in different layers of the canopy (Table 1). The canopy of *P. sylvestris* was also measured at three different layers during the fall to winter transition. However, this free-standing tree was exposed in the fall and winter to a very similar light environment within different layers. Thus, no vertical gradient in light or photosynthetic acclimation was observed within different layers, but seasonal photosynthetic downregulation and upregulation processes were (data not shown). Photosynthetic efficiency also depends on the fraction of absorbed PPFD (Monteith 1972). In the present study, we have assumed that the leaf absorbance of the investigated species remained approximately constant. This assumption may not be true during the leaf senescence period (Schultz 1996) and during the seasonal downregulation and upregulation processes. This potential error was, however, suggested to be rather small, in the range of ~5% (Oliveira and Penuelas 2004).

Overall, the study has demonstrated that the LIFT approach is a valuable tool to assess the diurnal and seasonal dynamics of photosynthesis in inaccessible canopies as it provides a rather unique way to study the spatial and temporal variation of photosynthetic parameters during seasonal plant growth and development. Still, interpretation of the LIFT narrow-leaf canopy measurements is challenging and requires more extensive field observations combined with an advanced canopy radiative transfer modeling exercise.

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Conflict of interest

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